**[Frontiers in Bioscience 9, 359-369, January 1, 2004]**

# ANGIOTENSIN II, PPAR-GAMMA AND ATHEROSCLEROSIS

**Ulrich Kintscher [1], Christopher J. Lyon [2] and Ronald E. Law [2]**

*[1] Institute of Pharmacology and Toxicology, Charité Hospital, Humboldt-University Berlin, D-10117 Berlin, Germany and [2] University of California, Los Angeles, School of Medicine, Department of Medicine, Division of Endocrinology, Diabetes and Hypertension, Los Angeles, CA 90095, USA*

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## 1. ABSTRACT

Atherosclerosis is a complex, chronic disease state that usually arises from the converging action of several pathogenic processes, including hypertension, hyperlidemia, obesity and insulin resistance. Significantly, due to the increasing incidence of type 2 diabetes worldwide, several aspects of the renin-angiotensin system, including the capacity for angiotensin II synthesis and binding are increased in human and animal models of type II diabetes, and potentiate vascular lesion formation. Angiotensin II, an important vasoactive peptide of the renin-angiotensin system, profoundly accelerates atherosclerosis in animal models of diabetes. Conversely, in both human and animal studies, inhibition of angiotensin II synthesis or activity has been shown to significantly reduce atherosclerosis and cardiovascular mortality. Cardiovascular protection is independent of blood pressure and baseline activity of the renin-angiotensin system, suggesting an important and direct role for the vascular renin-angiotensin system in atherosclerotic progression. Angiotensin II appears to accelerate atherosclerosis through activation of several distinct signal transduction pathways, and *via* these mechanisms can function as a vascular growth and migration factor, a pro-inflammatory cytokine and an oxidative stress agent. Thiazolidinediones, a class of oral insulin-sensitizing agents in broad clinical use for the treatment of type 2 diabetes, have been shown to ameliorate cardiovascular disease in animal trials and clinical studies. Thiazolidinediones also appear to regulate angiotensin II signaling at multiple levels, significantly reducing the expression of the angiotensin II type 1 receptor and repressing signal transduction through this receptor to suppress vascular remodeling, lesion formation, and oxidative stress.

## 2. INTRODUCTION

Angiotensin II (AngII) is a major proatherogenic factor through its actions to elevate blood pressure, induce inflammation in the vessel wall, and stimulate the growth and movement of vascular cells. (1-4). Physiological effects of AngII are mediated by its binding to and activation of two separate seven transmembrane domain receptors, designated as the AngII type-1 (AT1) and type-2 (AT2) subtypes (5). Tissue levels of AngII are regulated through the activity of the renin-angiotensin system (RAS) (6). Peroxisome proliferator activated receptor gamma (PPAR-gamma) is a member of the nuclear hormone receptor superfamily that is expressed in all vascular cells relevant to the development of atherosclerosis: vascular smooth muscle cells (VSMC), endothelial cells, and monocytes/macrophages (7-10). Activation of PPAR-gamma in mouse models of atherosclerosis significantly attenuates lesion development (11-15). Inhibition of atherogenesis by PPAR-gamma may result from its effect to block the proliferation of VSMCs and/or the migration of VSMCs and monocytes (16-21). Interactions between AngII and PPAR-gamma signaling in the vasculature are poorly understood but may have an important influence on atherosclerosis. Understanding such interactions will have important clinical ramifications as pharmacologic blockade of the RAS and administration of insulin-sensitizing PPAR-gamma ligands are widely used to treat persons with type 2 diabetes, who have increased risk for atherosclerosis.

## 3. THE ROLE OF ANGIOTENSIN II IN ATHEROSCLEROSIS

Historically, the renin-angiotensin system (RAS) has been known as a regulatory system involved in blood

pressure control, water and sodium homeostasis and neurohumoral modulation (22, 23). Blockade of the RAS, through angiotensin I converting enzyme (ACE) inhibition or AT1 receptor (AT1R) antagonism, effectively improves pathological conditions such as hypertension, renal disease and congestive heart failure (4, 24). In the past decade, data from clinical and experimental studies have accumulated suggesting a direct role for the RAS in the pathogenesis of atherosclerosis.

### 3.1. Clinical Data

The Heart Outcomes Prevention Evaluation (HOPE) trial demonstrated forcefully the pathophysiological link between AngII and human atherosclerotic disease (25). The HOPE study randomized 9297 high-risk patients who had clinical evidence of vascular disease, or diabetes and one other cardiovascular risk factor, to either the ACE-inhibitor ramipril or placebo. The primary endpoint after a mean follow-up of 4.5 years was a composite of myocardial infarction, stroke or death from cardiovascular causes. A significant 21% decrease in the primary endpoint was observed with ACE-inhibition. Cardiovascular events, such as myocardial infarction and stroke, were clearly reduced in the ramipril group. Ramipril only modestly reduced blood pressure (3.5/ 1.5mmHg), which could not account completely for the risk reduction observed. Therefore, this data suggests that inhibition of an endogenous RAS directly modifies fundamental pathological processes during atherogenesis in the vascular wall.

A substudy of the HOPE trial – the Study to Evaluate Carotid Ultrasound Changes in Patients Treated with Ramipril and Vitamin E (SECURE) – randomly assigned a total of 732 patients (vascular disease, or diabetes and at least one other risk factor) to receive ramipril and vitamin E or a matching placebo (26). Effects of ACE inhibition on atherosclerosis progression were studied by using duplicate B-mode carotid ultrasound to assess intima-media thickness of the carotid artery. Progression of atherosclerosis was reduced by 37% after 5 years of treatment with ramipril, and was largely independent of blood pressure reduction. These data clearly indicate that RAS blockade by ACE inhibition exerts direct beneficial effects on the vascular atherosclerotic process.

Direct favorable effects of RAS inhibition on cardiovascular pathology are further supported by the Losartan Intervention For Endpoint Reduction in Hypertension Study (LIFE), in which the AT1R antagonist losartan was compared to the β-blocker atenolol for their abilities to reduce cardiovascular events in hypertensive participants with left ventricular hypertrophy (27). Losartan reduced cardiovascular morbidity and death more than atenolol given a similar reduction in blood pressure. This benefit may result from increased protection against direct pathological effects of the RAS on cardiovascular structures.

ACE inhibition does not reduce all sources of AngII, since chymase and cathepsin G are also able to catalyze the hydrolysis from angiotensin I to AngII (28). Thus a combination of ACE inhibition and AT1R blockade, to prevent AT1R binding of residual AngII, should enhance the beneficial effects of each drug alone. Beneficial effects of the combination (AT1R-blocker and ACE-inhibitor) have already been shown in patients with heart failure (29). Ongoing trials (e.g. –The ongoing Telmisartan Alone and in Combination with Ramipril Global Endpoint Trial) are currently evaluating whether this combination might also impact atherosclerotic cardiovascular events (30).

In summary, the data from clinical studies suggest a direct pathophysiological role of the RAS, independent of blood pressure control, in the development and progression of atherosclerotic cardiovascular diseases.

### 3.2. Animal Model Data

Infusion of AngII into a hypercholesterolemic atherosclerosis-prone mouse model dramatically accelerates the rate of lesion formation (1, 31). In two separate studies, aortic surface lesions increased by ~20-fold after 4- or 8-week subcutaneous infusions of AngII into apoE-deficient ($apoE^{-/-}$) mice (1, 31). Elevation of blood pressure to a similar degree with norepinephrine, however, produced only one-quarter of the atherogenic stimulus provided by AngII (1). Direct vascular actions of AngII, rather than pressor effects, likely account for the majority of AngII's proatherogenic activity.

Diabetes is a strong cardiovascular risk factor, and RAS-associated mechanisms may explain much of the severity of this response. ACE activity is significantly enhanced in both diabetic subjects and animal models of type 2 diabetes (32). AT1R expression is also significantly upregulated in the vasculature of diabetic rat models (33, 34), suggesting that elevated AT1R expression could potentiate the AngII atherosclerotic activity in type 2 diabetes. Finally, at the signal transduction level, diabetes-associated hyperglycemia has been shown to induce VSMC nuclear factor (NF)-kappaB activation (35), which is known to regulate several inflammatory mechanisms involved in atherosclerosis, including AT1R signal transduction (36).

Inhibition of the RAS has also been shown to attenuate atherosclerosis in a number of experimental animal models. In $apoE^{-/-}$ mice, the ACE-inhibitor fosinopril decreased the average size of atherosclerotic lesions by more than 70% after a three-month treatment period (37). Similarly, in $apoE^{-/-}$ mice made diabetic with streptozotocin, ACE inhibition with perindopril suppressed the resulting diabetes-associated accelerated atherosclerosis and elevated aortic ACE expression (32). ACE inhibitors have proven to be equally efficacious in suppressing atherosclerosis in Watanabe hypercholesterolemic rabbits and in minipigs (38, 39). Because ACE inhibitors can elevate levels of the vasodilator bradykinin, in addition to decreasing AngII formation, it has been difficult to unequivocally ascribe their antiatherogenic activity to a blockade of the RAS. To address their uncertainty, several investigators have examined the effect of AT1R blockers (ARBs) on atherogenesis.

Similar to ACE inhibitors, ARBs have potent *in vivo* antiatherogenic activity. In cholesterol-fed cynomolgus monkeys, the ARB losartan substantial attenuated atherosclerosis across the aorta (40). Attenuation of atherosclerosis in atherosclerosis-prone apoE$^{-/-}$ mice and hypercholesterolemic rabbits has also been observed using, respectively, the ARBs irbesartan and valsartan (41, 42). Acceleration of atherosclerosis by administration of AngII and its inhibition by both ACE inhibitors and ARBs underscore the potent proatherogenic effect of AT1R-mediated AngII signalling on the vessel wall.

### 3.3. Cellular mechanisms

#### 3.3.1. Angiotensin II as a vascular growth and migration factor

Inflammatory processes play a key role in the development and progression of atherosclerosis (43). The hypothesis that AngII serves as a proinflammatory protein inducing an inflammatory reaction in the vessel wall and, thereby, initiating the atherosclerotic process has been supported by several in-vitro and in-vivo studies. Proinflammatory actions of AngII have been described in all vascular cells.

The first step in vascular lesion formation is the recruitment and transmigration of monocytes into the vascular subendothelium, a multifactorial process that is regulated by AngII at multiple levels (44). Angiotensin II potently induces the expression of adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and E-selectin, on endothelial cells, resulting in enhanced monocyte binding to the vascular endothelium (45-47). Monocyte chemotactic protein-1 (MCP-1) is a chemokine that attracts adherent monocytes to the site of the vascular lesion. Deficiency of MCP-1 or the major MCP-1 receptor CCR2 reduces atherosclerosis in apoE$^{-/-}$ mice (48, 49). Angiotensin II treatment stimulates the expression of MCP-1 in vascular smooth muscle cells (15). In addition, AngII itself serves as a chemotactic protein for human monocytes, enhancing the extravasation of these cells into the vessel wall (50).

The next step of atherogenesis is the accumulation of cholesterol into vascular tissue macrophages and subsequently the formation of foam cells (51). The oxidative modification of cholesterol/ LDL cholesterol in the vessel wall is a prerequisite for its uptake by macrophages (51). Angiotensin II directly promotes the oxidation of LDL cholesterol and stimulates its uptake by macrophages through induction of the scavenger receptor CD36 (52). Moreover, oxidized LDL induces potentially proatherosclerotic effects in endothelial cells via binding to the endothelial lectin-like oxidized LDL receptor-1 (LOX-1) (53). Binding of oxidized LDL to LOX-1 leads to the impairment of endothelial nitric oxide formation, induction of adhesion molecule expression and apoptosis (53). Angiotensin II also induces LOX-1 expression in endothelial cells exponentiating the proatherosclerotic effects of oxidized LDL (54).

A later step in atherogenesis is the induction of vascular smooth muscle cell (VSMC) proliferation and migration by growth factors and cytokines present in the atherosclerotic lesion (38, 55). We and others have demonstrated that AngII is a potent inducer of VSMC growth and migration, thereby contributing to the progression of lesion formation (56, 57). Interleukin-6 (IL-6) is one of the major cytokines expressed after vascular injury and is secreted by monocytes and VSMC (58). IL-6 has been demonstrated to promote VSMC proliferation through local paracrine actions (59). Its expression is prominently upregulated by AngII in VSMC enhancing AngII proliferative effects on lesional VSMCs (60).

Recent work has indicated a role for cycloxygenase-2 (COX-2) in VSMC growth and migration in response to AngII and inflammatory cytokines. AngII-stimulated COX-2 expression in VSMC is attenuated by AT1R antagonism or ERK MAPK inhibition (61-63), and AngII-mediated VSMC cell proliferation and migration are blocked by treatment with the COX-2 selective inhibitors NS-398 and nimesulide (61-63). AngII-stimulated COX-2 expression in VSMC is also attenuated by PPAR-alpha and PPAR-gamma ligands (63). Similar results were observed in human and rat VSMC, however, AngII appears to stimulate COX-2 mRNA expression in human VSMC (63), and extend COX-2 mRNA half-life in rat VSMC (61).

Both COX-2 and inducible nitric oxide synthase (iNOS) are induced by cytokines in several *in vitro* and *in vivo* systems, which has lead to the theory of cross talk between these two pathways. IL-1beta, however, induces both iNOS and COX-2 expression in rat VSMC, but only COX-2 expression in human VSMC (64). Nitric oxide has also been reported to influence the expression of COX-2. However, reports of a potential regulatory interactions between NO and COX-2 are contradictory. Nitric oxide has been reported to decrease COX-2 expression and activity by several groups (65, 66), while others, using similar experimental models, have found NO to increase the activity and expression of COX-2 (67, 68).

#### 3.3.2. Angiotensin II as a pro-inflammatory cytokine

Angiotensin II mediates its physiologic effects by binding to two highly-specific transmembrane receptors, referred to as the AT1R and AT2R subtypes, which are both expressed on vascular cells (23). Most of the known effects of AngII are related to AT1R activation, however, recent studies have indicated a functional role for the AT2R in AngII-mediated atherosclerotic processes. It has been hypothesized that the functions of AT1R and AT2R stimulation are mutually antagonistic (23, 69). Given the pro-inflammatory actions of AT1R activation, a potential anti-inflammatory role of the AT2R in these processes has been recently studied in a model of vascular inflammation using AT2R$^{-/-}$ mice (70). Treatment with the AT1R antagonist Valsartan led to a significant decrease in MCP-1, TNF-alpha, IL-6 and IL-1beta production, and infiltration of inflammatory cells in cuff-injured arteries (70). Interestingly, valsartan treatment was less effective in the inhibition of inflammation in AT2R$^{-/-}$ mice, indicating that AT2R activation is mediating, at least in part, the anti-inflammatory effects of AT1R blockade (70). The anti-inflammatory actions of the AT2R are further supported by

the enhanced inflammatory reaction observed in $AT2R^{-/-}$ mice after cuff-injury. Additional studies are required in the future to delineate the function of the AT2R in AngII-induced vascular inflammation and atherogenesis.

Further downstream mechanisms by which AngII induces vascular inflammation have been studied extensively. A key regulator of inflammatory gene regulation is the transcription factor NF-kappaB (13, 71). NF-kappaB is a highly inducible DNA-binding protein sequestered in the cytoplasm by association with one of several isoforms of the NF-kappaB inhibitor protein, I-kappaB. Upon activation by various cytokines and growth factors NF-kappaB is released from I-kappaB and translocates to the nucleus. NF-kappaB regulates an array of gene responses, many of which determine the degree of the inflammatory response (36, 71). Angiotensin II has pleiotropic actions at multiple levels of the NF-kappaB signalling pathway. Angiotensin II induces the translocation of cytoplasmic NF-kappaB to the nucleus by stimulating proteolysis of I-kappaB (60). Angiotensin II also enhances the binding of NF-kappaB subunits to DNA recognition sites in the promoter regions of target genes (60, 72). Several studies have shown that AngII-mediated IL-6, VCAM-1 and MCP-1 expression is regulated by NF-kappaB activation, demonstrating the functional importance of the NF-kappaB pathway to AngII induced vascular inflammation (2).

In summary, the pro-inflammatory actions of AngII in the vessel wall provide a logical pathophysiological scheme for direct proatherosclerotic effects of AngII.

### 3.3.3. Angiotensin II as an agent of vascular oxidative stress

Atherosclerosis as an inflammatory disease is amplified by vascular oxidative stress, a process that results in the formation of so-called reactive oxygen species (ROS) (73). The reduction of molecular oxygen results in multiple ROS, including superoxide ($O_2^{\cdot -}$), hydroxyl radicals ($HO^{\cdot}$) and hydrogen peroxide ($H_2O_2$). The major source of ROS in the vessel wall is the membrane-associated enzyme NADPH oxidase, which consists of multiple subunits (gp91phox, p22phox, p47phox, p67phox) that are differently expressed in endothelial cells, VSMCs, and fibroblasts (74). Increased NADPH oxidase activity results in excessive production of ROS, leading to oxidative modification of DNA and protein, lipid oxidation and activation of redox-sensitive genes.

Angiotensin II potently activates vascular NADPH oxidase, via AT1R stimulation, by increasing p22phox subunit expression (75). In addition, AngII also enhances the expression of other NADPH oxidase components, such as rac1 and the gp91phox homologue nox-1 (76, 77). Activation of NADPH oxidase by AngII results in an increased production of ROS in the vessel wall (78). ROS are central modulators of AngII-induced proatherosclerotic responses including the described inflammatory actions, and inactivation of NO by AngII-induced ROS plays a pivotal role in the initiation of atherosclerosis (78).

Dysregulation of the balance between vascular AngII and NO activities leads to endothelial dysfunction, the first step in lesion formation, decreasing vascular vasodilatory capacity and increasing the activity of monocytes/macrophages and platelets on the vessel wall. Nitric oxide decreases VSMC growth (79), stimulates endothelial cell growth and survival (80, 81), decreases platelet adherence to the endothelium (82), and suppresses monocyte adhesion, invasion and accumulation within the vessel wall (83).

Angiotensin II also appears to alter NO availability through regulation of VSMC iNOS expression. Reports detailing AngII effects on iNOS expression, however, present a complicated picture. SHR rats, a rat model of hypertension, demonstrate significantly elevated vascular iNOS mRNA and protein expression, relative to normotensive controls, which is reversed upon treatment with the ARB candesartan (84). An earlier *in vitro* study, however, did not detect AngII stimulation of iNOS expression in rat VSMC, and instead found that AngII suppresses IL-1beta stimulated iNOS expression *via* an AT1R-dependent mechanism (85). In humans, ACE administration was found to stimulate VSMC iNOS and AT1R expression, while decreasing vascular ACE (86).

A second consequence of vascular ROS induction by AngII is the induction of redox-sensitive gene expression. Endothelial VCAM-1 is a redox-sensitive gene that has been shown to be upregulated by AngII (45). Recently, it has been demonstrated that AngII-induced intracellular $H_2O_2$ production followed by NF-kappaB activation is required for VCAM-1 induction in rat aortic endothelial cells, clearly corroborating the relevance of oxidative stress in these processes (45). Other redox-sensitive genes involved in AngII-induced vascular inflammation are ICAM-1 and MCP-1 (78).

Production of $O_2^{\cdot -}$ in the vessel wall by AngII also promotes the oxidative modification of LDL cholesterol facilitating its uptake into lesional macrophages (87).

Another redox mechanism of AngII-induced atherogenesis is based on the function of NADPH-derived ROS as second messengers. It has been shown that AngII-induced activation of the mitogen-activated protein kinases p38 kinase and the cell survival kinase Akt (protein kinase B) in VSMCs involves the activation of NADPH-oxidase and production of ROS (88, 89). The p38 kinase and Akt are central regulators of VSMC growth and apoptosis, both processes which determine the number of lesional VSMC. The parallel increased production of ROS enhances AngII-induced downstream signalling resulting in a boost of AngII-mediated VSMC growth and promoting the progression of atherosclerosis.

Taken together, the induction of vascular oxidative stress by AngII is an additional proatherogenic stimulus, which is amplifying the pro-inflammatory actions of AngII. Angiotensin II´s oxidative properties together with its inflammatory effects provide striking mechanistic

evidence for a central pathophysiological role of this peptide in the atherosclerotic process.

## 4. PPAR-GAMMA AND ATHEROSCLEROSIS

Atherosclerosis is the major macrovascular complication of type 2 diabetes and the principal cause of mortality in that population (90-92). Recent studies suggest that metabolic abnormalities related to the Insulin Resistance Syndrome may trigger vascular damage that contributes importantly to the increased risk of atherosclerosis associated with diabetes (93-95). This emerging link between insulin resistance and atherosclerosis has focused attention on potential vascular benefits to be derived from insulin-sensitizers used to treat type 2 diabetes. Thiazolidinediones (TZDs) are oral insulin-sensitizers in broad clinical use to enhance insulin-stimulated glucose uptake into peripheral tissues (20, 96-99). Through pleiotropic activities to improve cardiovascular risk factors associated with the Insulin Resistance Syndrome and exert direct antiatherogenic effects on vascular cells, TZDs have the potential to retard the atherosclerotic process (8).

TZDs are ligands for the nuclear receptor, peroxisome proliferator activated receptor gamma (PPAR-gamma). All of the principal cell types presents in atherosclerosis lesions express PPAR-gamma, including intimal macrophages and vascular smooth muscle cells (16, 17, 19, 100). Direct vascular effects of TZDs, therefore, likely result from activation of PPAR-gamma in the arterial wall. Rosiglitazone and pioglitazone are the two currently available TZDs used clinically to ameliorate insulin resistance. Direct vascular effects of rosiglitazone, pioglitazone, and other nonclinical PPAR-gamma ligands that are relevant to the development of atherosclerosis include: inhibition of VSMC growth (19, 20); inhibition of VSMC and monocyte migration (16-19); and attenuation of proinflammatory responses by macrophages and T lymphocytes (101-103).

In addition to their direct effects on vascular cells, TZD PPAR-gamma ligands also increase HDL and reduce triglycerides (104, 105). Both rosiglitazone and pioglitazone modestly lower blood pressure in animal models of hypertension (106, 107), while rosiglitazone has been reported to exert a similar effect in humans (108). Reduced levels of circulating plasminogen activator inhibitor-1 (PAI-1), small dense LDL particles and C-reactive protein (CRP) have also been documented after treatment of type 2 diabetic subjects with troglitazone (109). Favorable effects of PPAR-gamma ligands on the vasoactive, inflammatory, thrombotic and dyslipidemic milieu as evidenced in clinical trials further buttress their promise to retard or prevent atherosclerosis.

Cardiovascular outcome studies in humans with PPAR-gamma ligands (rosiglitazone and pioglitazone) analogous to the HOPE trial for ACE inhibitors, will ultimately determine if this class has clinical antiatherogenic activity. Encouraging, although not necessarily predictive, results are provided by studies examining the effect of PPAR-gamma ligands in mouse models of atherosclerosis. PPAR-gamma ligands inhibit atherogenesis in both LDL receptor-deficient ($LDLR^{-/-}$) and $apoE^{-/-}$ mice (12-15). When $LDLR^{-/-}$ mice are administrated a high fat or high-fructose diet, they become hypercholesterolemic and form early atherosclerotic lesions, i.e. fatty streaks in their vessels (13). On a high-fat diet, male $LDLR^{-/-}$ mice also become obese, insulin resistant and over time develop hyperglycemia (12, 13). In contrast, a high fructose diet does not trigger insulin resistance or cause weight gain in this strain (13). Troglitazone, the first TZD PPAR-gamma ligand used clinically until its withdrawal for hepatotoxicity, suppresses the atherosclerotic lesion formation by 30-40% in aortae of $LDLR^{-/-}$ male mice fed either a high fat or high fructose diet (13). In a separate study, rosiglitazone markedly reduced lesion size in the aortic root of male $LDLR^{-/-}$ mice fed a high fat atherogenic diet (12). Antiatherogenic effects were associated with a reduction in the accumulation of lesional macrophages and decreased vascular expression of several proinflammatory genes, including TNF-alpha and CCR2, the receptor for MCP-1 (12, 110, 111). Attenuation of atherosclerosis by PPAR-gamma ligands appeared to be uncoupled from any effect to normalize insulin resistance or improve the dyslipidemia in $LDLR^{-/-}$ mice (12, 13). Although PPAR-gamma clearly impacts many aspects of arterial wall biology and metabolism in humans, results from mouse models implicate anti-inflammatory effects as a major mechanism for its antiatherogenic activity.

## 5. PPAR-GAMMA AND ANGIOTENSIN II INTERACTIONS IN THE VESSEL WALL

Regulation of AngII signalling by PPAR-gamma ligands can be inferred from their effect to modestly lower blood pressure. PPAR-gamma can regulate AngII signaling at several levels. Several different groups have shown that PPAR-gamma ligands can repress transcription of the AT1R gene, possibly by interfering with SP1 promoter elements (112-114). Other studies have demonstrated that PPAR-gamma ligands can block AngII signaling downstream of the AT1R. Conversely, elevated AngII levels, associated with increased atherosclerotic risk, may attenuate the action of endogenous PPAR-gamma ligands in the vasculature, since AngII-infusion has been shown to downregulate PPAR-gamma and PPAR-alpha mRNA and protein expression throughout the aortae of an $apoE^{-/-}$ mouse model of atherosclerosis (115).

PPAR-gamma ligands block AT1R-mediated activation of ERK MAPK, which is required for AngII stimulation of VSMC growth and migration (116-119). Activation of ERK MAPK may mediate many of AngII's proatherogenic effects in the vessel wall (120). It is currently unknown whether PPAR-gamma ligands impact increased oxidative stress in the vasculature mediated by AngII.

In preliminary studies, PPAR-gamma ligands were effective in inhibiting AngII-accelerated atherosclerosis in $LDLR^{-/-}$ mice (121, 122). Rosiglitazone, pioglitazone, and a non-TZD PPAR-gamma ligand all

reduced atherosclerosis by ~60%. These effects occurred in the absence of any significant change in cholesterol, triglycerides, insulin, glucose, and blood pressure. The failure of PPAR-gamma ligands to lower blood pressure was somewhat unexpected because of their reported *in vitro* effect to lower AT1R levels in VSMC (112-114). Attenuation of lesion formation by PPAR-gamma ligands did correlate with a pronounced downregulation of the proinflammatory transcription factor Egr-1 and a concomitant reduction in several Egr-1 target genes, including TNF-alpha, ICAM-1, and MCP-1 (121, 122). AngII is also a potent activator of NF-kappaB and AP-1, two other transcription factors that function analogously to Egr-1 as master orchestrators of the inflammatory response (102, 123). PPAR-gamma can also repress transcription of many NF-kappaB- and AP1-regulated genes that promote inflammation, a process that likely also contributes to the observed attenuation of AngII-accelerated atherosclerosis by PPAR-gamma ligands.

## 6. CONCLUSION

AngII has emerged as a major culprit during atherogenesis by elevating blood pressure, increasing oxidative stress, and provoking an inflammatory response. In addition to their important ability to ameliorate insulin resistance, PPAR-gamma ligands may also protect against atherosclerosis as revealed by studies in mouse models. PPAR-gamma ligands may constitute a unique promising class of therapeutics for treating diabetes-associated cardiovascular disease through their pleiotropic activity to target AngII-mediated processes in the vessel wall, normalize metabolic abnormalities of the Insulin Resistance Syndrome, and suppress vascular inflammation.

## 7. ACKNOWLEDGEMENTS

This work was supported by NIH/National Heart, Lung and Blood Institute Grant 1P01 HIG0030-01A1 to Willa A. Hsueh.

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**Abbreviations:** AngII: angiotensin II, PPAR-gamma: peroxisome proliferator activated receptor gamma, TNF-alpha: tumor necrosis factor alpha, NF-kappaB: nuclear factor kappaB, I-kappaB: inhibitor of nuclear factor kappaB, TZD: thiazolidinedione, AT1R: angiotensin II type-1 receptor, VSMC: vascular smooth muscle cells, RAS: renin-angiotensin system, ACE: angiotensin I converting enzyme

**Key words:** Angiotensin, atherosclerosis, Vessel, Endothelium, PPAR-gamma, Review

**Send correspondence to:** Ronald E. Law, Ph.D., UCLA School of Medicine; Division of Endocrinology, Diabetes and Hypertension, Warren Hall, Second Floor, Suite 24-130, 900 Veteran Avenue, Box 957073, Los Angeles, CA 90095, USA, Tel: 310-794 7555, Fax: 310-794 7654, E-mail: rlaw@mednet.ucla.edu